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Health and Environmental Effects Profile for 3,3'-Dimethoxybenzidine

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR 3,3'-DIMETHOXYBENZIDINE

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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1985.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Reference doses (RfDs) for chronic exposures. An RfD is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, RfDs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA and CERCLA dockets.

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EXECUTIVE SUMMARY

3,3'-Dimethoxybenzidine is a colorless crystalline compound at ambient temperatures that turns violet upon exposure to air. It is a weak base that exhibits the general characteristics of primary aromatic amines (IARC, 1974). As of 1978, the only U.S. manufacturer of 3,3'-dimethoxybenzidine was Upjohn Co., which manufactures "very small" amounts of this compound once or twice per year (Powell et al., 1979). In 1983, 106,005 pounds of this compound were imported (USITC, 1984). It is estimated that >75% of the 3,3'-dimethoxybenzidine manufactured is used as an intermediate in the production of dyes and pigments for coloring leather, paper, plastics, rubber and textiles (Powell et al., 1979; IARC, 1974). Approximately 25% of the 3,3'-dimethoxybenzidine is used to manufacture 3,3'-dimethoxy-4,4'-diphenylene diisocyanate, an ingredient in isocyanate adhesives and a component of polyurethane elastomers (Powell et al., 1979; IARC, 1974).

3,3'-Dimethoxybenzidine is a weak base and as such, may be protonated and form salts that are more soluble than the parent compound. Variations in the behavior of 3,3'-dimethoxybenzidine in the environment may result from protonation. If released to water, 3,3'-dimethoxybenzidine is likely to be resistant to hydrolysis. Potential exists for significant photolysis of 3,3'-dimethoxybenzidine. The importance of biodegradation is not clear since the limited available data on the biodegradability of 3,3'-dimethoxybenzidine in water were conflicting (Baird et al., 1977; Brown and Laboureur, 1983; Kawasaki, 1980). Adsorption and covalent binding to suspended solids and sediments may be significant. Bioaccumulation in aquatic organisms and volatilization from water should not be significant removal processes. If released to air, vapor phase 3,3'-dimethoxybenzidine

is predicted to react with hydroxyl radicals and has an estimated half-life of 6.52 hours at 25°C (U.S. EPA, 1986d). Direct photolysis in the troposphere is also a potentially significant removal mechanism. Reaction with ozone is not likely (U.S. EPA, 1986d). If released to soil, 3,3'-dimethoxybenzidine is likely to be resistant to hydrolysis (Lyman et al., 1982). 3,3'-Dimethoxybenzidine may be immobilized in soil by physical adsorption and covalent binding and may be mobilized from protonation in acidic soils. Volatilization from wet and dry soil surfaces should not be significant. Soil-catalyzed oxidation of 3,3'-dimethoxybenzidine may be the most significant reaction of this chemical and the half-life of this reaction may be <1 day (U.S. EPA, 1980).

Monitoring data regarding 3,3'-dimethoxybenzidine could not be located in the available literature. 3,3'-Dimethoxybenzidine may be released to the environment in the effluent from dye manufacturing and processing plants that do not adequately treat their wastewater (U.S. EPA, 1980). Loss of 3,3-dimethoxybenzidine during its production is ~1.0-2.0% and loss during dye and pigment manufacturing is ~0.5-1.3% (including free base, unreacted tetrazonium compound and soluble by-product) (Lapp et al., 1981). Release to the environment in airborne particulates from dye manufacturing and processing plants may also occur. Dermal exposure to the general population from consumer use of packaged dyes and pigments for home use derived from 3,3'-dimethoxybenzidine, as well as products treated with these compounds, may be possible (U.S. EPA, 1980).

3,3'-Dimethoxybenzidine is absorbed after oral or dermal exposure. Rats treated orally with ^{14}C-3,3'-dimethoxybenzidine excreted ~35% of the dose of radioactivity in the urine and ~50% in the feces in 3-8 days (Bowman et al., 1982; Rodgers et al., 1983). Experiments in bile-exteriorized rats

treated intravenously with ^{14}C-3,3'-dimethoxybenzidine indicated that biliary excretion of radioactivity was extensive and accounted for most of the fecal excretion. Of the biliary radioactivity ~33% was reabsorbed. Therefore, absorption of 3,3'-dimethoxybenzidine by the gastrointestinal tract was extensive. Rats treated dermally with ^{14}C-3,3'-dimethoxybenzidine absorbed ~28% of the applied dose of radioactivity at a half-life of 52.9 hours (Shah and Guthrie, 1983). The appearance of 3,3'-dimethoxybenzidine in the urine of workers occupationally exposed indicates that dermal absorption also occurs in humans (Meigs et al., 1954).

Following absorption, 3,3'-dimethoxybenzidine and its metabolites are widely distributed to tissues and organs, with the highest concentrations in the liver and gastrointestinal tract, probably because of biliary excretion and enterohepatic recirculation (Bowman et al., 1982; Rodgers et al., 1983; Shah and Guthrie, 1983). 3,3'-Dimethoxybenzidine was metabolized by N-acetylation, hydroxylation, O-demethylation and glucuronidation in rats (Rodgers et al., 1983). Unchanged parent compound was excreted in the urine and metabolites were excreted in the urine, feces and bile.

Rats treated by gavage with 3,3'-dimethoxybenzidine at 0.1-30 mg/rat/day, 5 days/week for 1 year and observed for an additional 6 months had a dose-related increase in total tumors (Hadidian et al., 1968). Small group sizes and incomplete reporting of control data preclude an adequate assessment of the results. Of 18 rats surviving treatment with 30 mg/rat/day, 3 days/week for 13 months, 4 developed tumors of the Zymbal gland, mammary gland or ovary (Pliss 1963, 1965). In hamsters treated at a dietary level of 0.1%, 1/30 males developed a bladder tumor, which was considered to be treatment-related because of the lack of occurrence in historic controls (Saffioti et al., 1967). Forestomach papillomas developed in 57% of

hamsters treated at a dietary level of 1% compared with 2% of controls (Sellakumar et al., 1969; OSHA/NIOSH, 1980). IARC (1982) considered these data to be sufficient evidence of carcinogenicity to animals. A chronic drinking water bioassay in rats is in progress (NTP, 1986). Epidemiology studies of 3,3'-dimethoxybenzidine alone were not available.

3,3-Dimethoxybenzidine generally has been positive in reverse mutation assays in Salmonella typhimurium with metabolic activation (Reid et al., 1984a,b; Anderson and Styles, 1978), for SCE in Chinese hamster ovary cells with and without metabolic activation (Galloway et al., 1985), for UDS in cultured rat hepatocytes (Probst et al., 1981), HeLa cells (Martin et al., 1978).

Pertinent data regarding the teratogenicity and reproductive effects of 3,3'-dimethoxybenzidine could not be located in the available literature as cited in the Appendix.

The toxicity of 3,3'-dimethoxybenzidine has not been adequately studied. Nonneoplastic lesions in the mammary gland, uterus, liver and testes were observed in rats treated by gavage with 0.1-30 mg/rat/day, 5 days/week for 52 weeks and observed for 6 months (Hadidian et al., 1968). Inadequate reporting of control data, small sample size and lack of statistical analysis of the data precluded assessing the significance of these results.

Hadidian et al. (1968) dosed groups of three male rats with 3.0, 10, 30, 100 and 300 mg/rat/day of 3,3'-dimethoxybenzidine by gavage 5 days/week for 4 weeks. All rats in the 100 and 300 mg/rat/day groups died. At 3 and 30 mg/rat/day, no rats died and at 10 mg one rat died. No control data were reported.

Pertinent data regarding effects of 3,3'dimethoxybenzidine on aquatic biota could not be located in the available literature as cited in the Appendix.

A q_1^* of 1.41×10^{-2} $(mg/kg/day)^{-1}$ was derived based on the incidence of forestomach papillomas in male and female hamsters fed 3,3'-dimethoxybenzidine in the diet in the study by Sellakumar et al. (1969) as reported by OSHA/NIOSH (1980). The water concentrations associated with increased lifetime risk at levels of 10^{-5}, 10^{-6} and 10^{-7} are 2.5×10^{-2}, 2.5×10^{-3} and 2.5×10^{-4} mg/ℓ, assuming a 70 kg man consumes 2 ℓ water/day. An F factor of 0.1 $(mg/kg/day)^{-1}$ was also calculated from the incidence of forestomach papillomas in hamsters. The F factor is <1 placing the chemical in Potency Group 3.

Although the animal studies are limited in defining carcinogenic activity because of the demonstrated mutagenicity of 3,3'-dimethoxybenzidine and because of its close structural relationship to benzidine compounds that are known carcinogens, the evidence for 3,3'-dimethoxybenzidine is upgraded to a sufficient level of animal data -- EPA Group B2. Thus, this compound is considered to be a probable human carcinogen.

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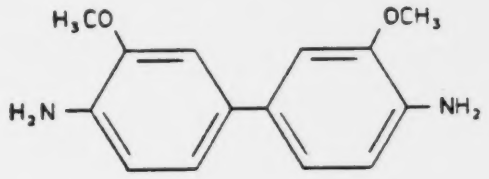
LIST OF ABBREVIATIONS

AHC	Alkaline hydrolyzable conjugates
BCF	Bioconcentration factor
CAS	Chemical Abstract Service
GC/MS	Gas chromatography/mass spectrometry
HPLC	High pressure liquid chromatography
K_{oc}	Soil sorption coefficient standardized with respect to soil organic carbon
K_{ow}	Octanol/water partition coefficient
MTD	Maximum tolerated dose
ppm	Parts per million
RQ	Reportable quantity
SCE	Sister chromatid exchange
UDS	Unscheduled DNA synthesis
UV	Ultraviolet

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

3,3'-Dimethoxybenzidine is also known as o-dianisidine, o-dimethoxybenzidine and 3,3'-dimethoxy-[1,1'-biphenyl]-4,4'-diamine. The structure, molecular weight, empirical formula and CAS Registry number are as follows:



Molecular weight: 244.30

Empirical formula: $C_{14}H_{16}N_2O_2$

CAS Registry number: 119-90-4

1.2. PHYSICAL AND CHEMICAL PROPERTIES

3,3'-Dimethoxybenzidine is a colorless crystalline compound at ambient temperatures that turns violet upon exposure to air (Werner, 1948). It is a weak base that has the general characteristics of primary aromatic amines (IARC, 1974), and is soluble in benzene, alcohol or ether (Windholz, 1983). Physical properties of 3,3'-dimethoxybenzidine are listed below:

Melting point:	137°C	Hawley, 1981
Boiling point:	429°C (estimated)	Neely and Blau, 1985
Flash point:	206°C	Hawley, 1981
Water solubility:	600 mg/ℓ at 25°C	U.S. EPA, 1986a
Log K_{ow}:	1.81 (calculated)	U.S. EPA, 1986b

1.3. PRODUCTION DATA

3,3'-Dimethoxybenzidine is produced commercially by reducing the methyl ether of o-nitrophenol (o-nitroanisole) with zinc dust and alkali to hydrazoanisole, which is subsequently rearranged with hydrochloric or sulfuric acid to 3,3'-dimethoxybenzidine (Powell et al., 1979; IARC, 1974). As of 1978, Upjohn Co. was the only U.S. manufacturer of 3,3'-dimethoxybenzidine and is reported to manufacture "very small" amounts once or twice per year (U.S. EPA, 1986c; Powell et al., 1979). In 1983, 106,005 pounds of this compound were imported through principal custom districts in the United States (USITC, 1984).

1.4. USE DATA

It has been estimated that >75% of the 3,3'-dimethoxybenzidine manufactured is used as an intermediate for the production of dyes and pigments (Powell et al., 1979; IARC, 1974). In 1980, it was used domestically to produce 36 azo dyes and 2 pigments (U.S. EPA, 1980; OSHA/NIOSH, 1980). The 3,3'-dimethoxybenzidine-based dyes and pigments are used for coloring leather, paper, plastics, rubber and textiles, and 3,3'-dimethoxybenzidine itself was formerly used for dyeing acetate nylon (IARC, 1974). Approximately 25% of 3,3'-dimethoxybenzidine is used to manufacture 3,3'-dimethoxy-4,4'-diphenylene diisocyanate, an ingredient in isocyanate adhesives and a component of polyurethane elastomers (Powell et al., 1979; IARC, 1974). 3,3'-Dimethoxybenzidine has been used to detect the presence of a number of metals, thiocyanates and nitrites (IARC, 1974)

1.5. SUMMARY

3,3'-Dimethoxybenzidine is a colorless crystalline compound at ambient temperatures that turns violet upon exposure to air. It is a weak base that exhibits the general characteristics of primary aromatic amines (IARC, 1974). As of 1978, the only U.S. manufacturer of 3,3'-dimethoxybenzidine was Upjohn Co., which manufactures "very small" amounts of this compound once or twice per year (Powell et al., 1979). In 1983, 106,005 pounds of this compound were imported (USITC, 1984). It is estimated that >75% of the 3,3'-dimethoxybenzidine manufactured is used as an intermediate in the production of dyes and pigments used for coloring leather, paper, plastics, rubber and textiles (Powell et al., 1979; IARC, 1974). Approximately 25% of the 3,3'-dimethoxybenzidine is used to manufacture 3,3'-dimethoxy-4,4'-diphenylene diisocyanate, an ingredient in isocyanate adhesives and a component of polyurethane elastomers (Powell et al., 1979; IARC, 1974).

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

3,3'-Dimethoxybenzidine is a weak base that may be protonated to form salts that are more water soluble than the parent compound. Protonation can alter the behavior of a compound in water and soil, for example, by decreasing volatilization as well as increasing or decreasing adsorption to soil, sediments and suspended solids in water.

2.1. WATER

2.1.1. Hydrolysis. Based on the structure of 3,3'-dimethoxybenzidine, this compound is likely to be resistant to hydrolysis (Lyman et al., 1982).

2.1.2. Oxidation. Pertinent data regarding the oxidation of this compound could not be located in the available literature as cited in the Appendix.

2.1.3. Photolysis. 3,3'-Dimethoxybenzidine in methanol strongly absorbs UV light in the environmentally significant range of wavelengths of >290 nm (Sadtler, n.d.). These data indicate that direct photolytic reactions may be significant in removing 3,3'-dimethoxybenzidine from water. Experimental data on the photolysis of this compound could not be located in the available literature as cited in the Appendix.

2.1.4. Biodegradation. Pertinent data regarding the biodegradation of 3,3'-dimethoxybenzidine were limited. An aerobic biodegradation screening study using 3 mg/ℓ of 3,3'-dimethoxybenzidine inoculated with yeast extract and activated sludge indicated that this compound is inherently biodegradable (Brown and Laboureur, 1983). After 6 hours, 20 mg/ℓ 3,3'-dimethoxybenzidine inoculated with activated sludge in a Warburg respirometer at 25°C was 98-100% depleted (Baird et al., 1977). 3,3'-Dimethoxybenzidine was, however, found to be resistant to microbial degradation using the MITI test as reported by Kawasaki (1980) and Sasaki (1978).

These authors reported that 100 ppm 3,3'-dimethoxybenzidine when inoculated with 30 ppm activated sludge at 25°C was <30% degraded after 2 weeks.

2.1.5. **Adsorption.** Based on observed covalent binding of primary amines to humates (Parris, 1980) and the estimated K_{oc} of 129-617 (Section 2.3.2.), physical adsorption and covalent bonding to suspended solids and sediments in water may be significant depending upon environmental conditions.

2.1.6. **Volatilization.** The pK_a of 3,3'-dimethoxybenzidine is not known but, because of the contribution of election donation by the methoxy groups to the benzene rings, it should be greater than that of benzidine. Since benzidine has a pK_1 of 4.66 and pK_2 of 3.57 (Perrin, 1965), it is likely that 3,3'-dimethoxybenzidine will protonate in water at pH <7 and should not volatilize significantly from water. The covalent binding with humates in water will also reduce volatility. The volatility may be higher at water pH >7 than at pH <7.

2.1.7. **Bioconcentration.** Results of the MITI test indicate that 3,3'-dimethoxybenzidine has a BCF of <100 (Kawasaki, 1980). The BCF for 3,3'-dimethoxybenzidine has also been estimated using the following recommended linear regression equations (Lyman et al., 1982):

$$\log BCF = 0.76 \log K_{ow} - 0.23 \qquad (2\text{-}1)$$

$$\log BCF = 2.791 - 0.564 \log S(mg/\ell) \qquad (2\text{-}2)$$

Based on the estimated log K_{ow} of 1.81 and a solubility of 600 mg/ℓ at 25°C, the BCF for 3,3'-dimethoxybenzidine has been calculated to be 14 and 17 from Equations 2-1 an 2-2, respectively. These BCF values indicate that 3,3'-dimethoxybenzidine should not bioconcentrate significantly in aquatic organisms. The regression equations for the estimation of the BCF were

developed for nonionic compounds and therefore are not suitable for estimating the BCF if the compound becomes protonated. It would be expected, however, that the protonated form would bioconcentrate even less than the unprotonated form.

2.2. AIR

2.2.1. Reaction with Hydroxyl Radicals. 3,3'-Dimethoxybenzidine in the vapor phase will react in the atmosphere with photochemically generated hydroxyl radicals. The hydroxyl reaction rate constant has been estimated to be 2.90×10^{-12} cm^3/molecule-sec at 25°C. Assuming an ambient hydroxyl radical concentration of 8.0×10^5 molecules/cm^3, the reaction half-life has been calculated to be 6.52 hours (U.S. EPA, 1986d).

2.2.2. Reaction with Ozone. 3,3'-Dimethoxybenzidine is not expected to react with ozone in the atmosphere (U.S. EPA, 1986d).

2.2.3. Photolysis. Since 3,3'-dimethoxybenzidine in methanol strongly absorbs UV light of wavelengths >290 nm (Sadtler, n.d.), direct photolysis is possible in the troposphere.

2.3. SOIL

2.3.1. Chemical and Microbial Degradation. No pertinent data regarding the chemical or microbial degradation of 3,3'-dimethoxybenzidine in soil were located in the available literature. Based on the molecular structure of 3,3'-dimethoxybenzidine, this compound is likely to be resistant to hydrolysis (Lyman et al., 1982). Benzidine, a congener of 3,3'-dimethoxybenzidine is reported to undergo rapid oxidation in clay soils with a half-life of <1 day (U.S. EPA, 1980). Therefore, it is expected that 3,3'-dimethoxybenzidine will undergo this soil-catalyzed oxidation and the half-life of this reaction may be <1 day.

2.3.2. **Adsorption.** Parris (1980) experimentally determined that primary amines covalently bind to humates (soil organic matter) initially by a rapid, reversible reaction followed by a slower irreversible binding process. The K_{oc} for 3,3'-dimethoxybenzidine has been calculated using the following linear regression equations (Lyman et al., 1982):

$$\log K_{oc} = -0.55 \log S(mg/\ell) + 3.64 \quad (2\text{-}3)$$

$$\log K_{oc} = -0.54 \log S(\text{mol fraction}) + 0.44 \quad (2\text{-}4)$$

Based on a water solubility of 600 mg/ℓ at 25°C, K_{oc} values of 129 and 617 were calculated from Equations 2-3 and 2-4, respectively. These K_{oc} values indicate that 3,3'-dimethoxybenzidine may be moderately mobile in soil (Swann et al., 1983); however, protonation in acidic soils and covalent binding with soil humic matter alter mobility. Thus, 3,3'-dimethoxybenzidine may be significantly immobilized in soil by physical adsorption and covalent binding and may be significantly mobilized in acidic soils by protonation.

2.3.3. **Volatilization.** Pertinent experimental data regarding volatilization of 3,3'-dimethoxybenzidine in soil could not be located in the available literature as cited in the Appendix. Based on the water solubility (600 mg/ℓ at 25°C), possible protonation in acidic soils and potential adsorption and covalent binding to soils (see Section 2.3.2.), volatilization from wet and dry soil surfaces is not expected to be significant.

2.4. SUMMARY

3,3'-Dimethoxybenzidine is a weak base and as such, may be protonated and form salts that are more soluble than the parent compound. Variations in the behavior of 3,3'-dimethoxybenzidine in the environment may result from protonation. If released to water, 3,3'-dimethoxybenzidine is likely to be resistant to hydrolysis. Potential exists for significant photolysis

of 3,3'-dimethoxybenzidine. The importance of biodegradation is not clear since the limited available data on the biodegradability of 3,3'-dimethoxybenzidine in water were conflicting (Baird et al., 1977; Brown and Laboureur, 1983; Kawasaki, 1980). Adsorption and covalent binding to suspended solids and sediments may be significant. Bioaccumulation in aquatic organisms and volatilization from water should not be significant removal processes. If released to air, vapor phase 3,3'-dimethoxybenzidine is predicted to react with hydroxyl radicals and has an estimated half-life of 6.52 hours at 25°C (U.S. EPA, 1986d). Direct photolysis in the troposphere is also a potentially significant removal mechanism. Reaction with ozone is not likely (U.S. EPA, 1986d). If released to soil, 3,3'-dimethoxybenzidine is likely to be resistant to hydrolysis (Lyman et al., 1982). 3,3'-Dimethoxybenzidine may be immobilized in soil by physical adsorption and covalent binding and may be mobilized by protonation in acidic soils. Volatilization from wet and dry soil surfaces should not be significant. Soil-catalyzed oxidation of 3,3'-dimethoxybenzidine may be the most significant reaction of this chemical and the half-life of this reaction may be <1 day (U.S. EPA, 1980).

3. EXPOSURE

Monitoring data regarding 3,3'-dimethoxybenzidine could not be located in the available literature as cited in the Appendix. 3,3'-Dimethoxybenzidine may be released to the environment in the effluent from dye manufacturing and processing plants that do not adequately treat their wastewater (U.S. EPA, 1980). Loss of 3,3-dimethoxybenzidine during its production is ~1.0-2.0% and loss during dye and pigment manufacturing is ~0.5-1.3% (including free base, unreacted tetrazonium compound and soluble by-product) (Lapp et al., 1981). Release to the environment in air-borne particulates from dye manufacturing and processing plants may also occur. Dermal exposure to the general population from consumer use of packaged dyes and pigments for home use derived from 3,3'-dimethoxybenzidine, as well as products treated with these compounds, may be possible (U.S. EPA, 1980).

4. PHARMACOKINETICS

4.1. ABSORPTION

Bowman et al. (1982) treated male rats with single oral doses that contained 14 µg of ^{14}C-3,3'-dimethoxybenzidine (uniformly ring-labeled) and 621 µg of unlabeled 3,3'-dimethoxybenzidine HCl. The vehicle was dilute aqueous HCl. After 192 hours, ~35 and 52% of the dose of radioactivity was excreted in the urine and feces, respectively. As 97% of the fecal radioactivity represented possible biliary metabolites, biliary excretion may have accounted for the high degree of fecal excretion. Therefore, absorption by the gastrointestinal tract may have been at least 85%. Similar results were obtained by Rodgers et al. (1983), who administered 50 or 1.08 mg/kg of ^{14}C-3,3'-dimethoxybenzidine (uniformly ring-labeled) in aqueous Emulphor 719 by gavage to male rats. Within 72 hours, the rats excreted ~33-35% and 48-52% of the dose of radioactivity in the urine and feces, respectively, regardless of dose. Rodgers et al. (1983) also injected rats intravenously with ^{14}C-3,3'-dimethoxybenzidine and found that 71% of the dose of radioactivity was excreted in the bile of bile-exteriorized rats in 72 hours, while intact rats excreted ~48% of the dose in the feces, which indicated that ~33% of the biliary radioactivity was reabsorbed by enterohepatic recirculation.

Shah and Guthrie (1983) applied 1 mg ^{14}C-3,3'-dimethoxybenzidine (uniformly ring labeled) in 0.2 mL acetone to rats in a 5-6 cm^2 area of the mid-back which had been clipped of hair. The rats were restrained from licking or abrading the treatment area. After 24 hours, ~28% of the radioactivity had disappeared from the site of application with a half-life of 52.9 hours, indicating substantial dermal absorption. After 24 hours, ~28% of the applied radioactivity was found in urine, feces, carcass, tissues and organs, confirming that the loss from skin represented absorption.

Meigs et al. (1954) detected 3,3'-dimethoxybenzidine in the urine of occupationally exposed workers. There was a direct correlation between the amount excreted and the degree of contamination of the workers' glove linings and underclothing. The air concentrations were not high enough to account for the urinary concentration, thus indicating that dermal exposure of humans results in absorption by this route.

4.2. DISTRIBUTION

Bowman et al. (1982) gave three Fischer 344 rats single oral doses of ^{14}C-3,3'-dimethoxybenzidine (see Section 4.1.) and observed the highest accumulation of radioactivity in the liver (0.568 µg equiv./g), bladder (0.147 µg equiv./g) and kidney (0.127 µg equiv./g). Tissue samples were taken 72 hours postexposure. Small amounts of radioactivity (0.006-0.395 µg equiv./g) were also found in the blood, brain, carcass, heart, intestinal tract and its contents, lung, muscle, spleen, stomach (and contents) and testes.

Rodgers et al. (1983) dosed male rats intravenously with ^{14}C-3,3'-dimethoxybenzidine. Three rats were sacrificed and exsanguinated 0.5, 1, 2, 4 and 8 hours/day and 3 days after exposure. Radioactivity was widely distributed to organs and tissues. After 2 hours, ~50% of the radiolabel was excreted from the bile into the intestinal tract and its contents. A high percentage of radiolabel, identified as 3,3'-dimethoxybenzidine and acetyl dimethoxybenzidine, was found in the stomach contents. The concentration of radiolabel in the liver was high at all time intervals, ranging from 9.49 to 1.26% at 0.5 and 72 hours, respectively. At 72 hours, the liver contained 45±2% of the radiolabel that remained in the body. A decline of radioactivity in all tissues was observed, especially during the first few hours of the experiment with the exception of the bladder, and

small and large intestines and their contents. After 72 hours, the total radioactivity was ~1/8 of the value measured at 0.5 hours.

Shah and Guthrie (1983) also assessed the distribution in rats of a dermally applied dose of 1 mg ^{14}C-3,3'-dimethoxybenzidine in 0.2 mℓ acetone (see Section 4.1.). Radioactivity was found in the heart, spleen, kidney, fat, bladder, ear, bone marrow, brain, muscle and lung and gastrointestinal tract at 1, 8 and 24 hours postexposure, but with the exception of the gastrointestinal tract the levels were generally <0.1% of the dose. Radioactivity in the liver ranged from 0.23-0.40% at 1, 8 and 24 hours postexposure. Radioactivity levels in the carcass were <5%.

4.3. METABOLISM

Rodgers et al. (1983) gave rats an oral dose of 50 mg/kg ^{14}C-3,3'-dimethoxybenzidine (uniformly ring-labeled) and identified urinary metabolites by GC/MS. Unchanged 3,3'-dimethoxybenzidine was the major urinary component. In addition, the following metabolites were identified and formed by N-acetylation, hydroxylation, O-demethylation and glucuronidation: hydroxy-dimethoxybenzidine, O-demethyl-dimethoxybenzidine, acetyl-dimethoxybenzidine, hydroxy-acetyl-dimethoxybenzidine, acetyl-O-demethyldimethoxybenzidine, diacetyl-dimethoxybenzidine, diacetyl-O-demethyldimethoxybenzidine and diacetyl-O-didemethyldimethoxybenzidine. A proposed pathway of 3,3'-dimethoxybenzidine metabolism is shown in Figure 4-1. The major biliary metabolite in rats injected intravenously was tentatively identified as 3,3'-dimethoxybenzidine-N-glucuronide. Acetyl-dimethoxybenzidine and at least 12 other metabolites were also present in bile. Measurement of diacetyldimethoxybenzidine in homogenates of intravenously treated whole rats revealed that ~16% of the initial dose was converted to diacetyl

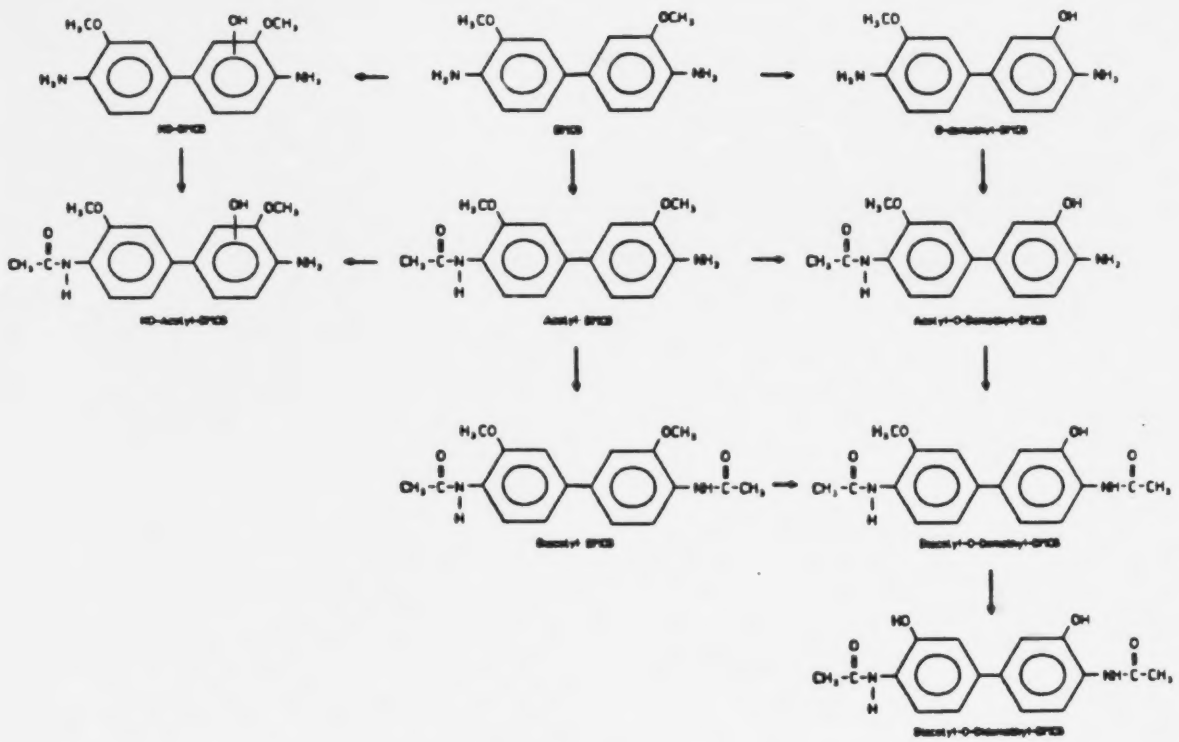


FIGURE 4-1

Proposed Pathway of Dimethoxybenzidine Metabolism

Source: Rodgers et al., 1983

dimethoxybenzidine at 30 minutes indicating that the 3,3'-dimethoxybenzidine was rapidly metabolized. Less than 2% of radioactivity was present as the parent compound.

4.4. EXCRETION

Bowman et al. (1982) (see Section 4.1.) observed that ~35 and 52% of an administered dose of radioactivity was excreted in the urine and feces, respectively, of rats within 192 hours of an oral dose of ^{14}C-3,3'-dimethoxybenzidine. Excretion by both routes reached a peak between 8 and 16 hours after exposure.

Rodgers et al. (1983) administered doses of 1.08 and 50 mg/kg ^{14}C-3,3'dimethoxybenzidine by gavage to two groups of three rats. Urine and feces were collected 24 hours/day for 3 days. Approximately 85% of radiolabel was excreted in the urine and feces after 3 days (~50% of the dose was observed in the feces). The percentage of radioactivity excreted in the urine and feces was independent of dose.

Rodgers et al. (1983) also studied the biliary excretion of 3,3'-dimethoxybenzidine by giving rats with exteriorized bile ducts intravenous injections of ^{14}C-3,3'-dimethoxybenzidine. After 72 hours, 71% of the administered dose of radioactivity was excreted in the bile with most of the excretion occurring from 0-12 hours. Since intact rats excreted ~48% of the radioactivity in the feces, ~33% of the biliary radiolabel was reabsorbed.

4.5. SUMMARY

3,3'-Dimethoxybenzidine is absorbed after oral or dermal exposure. Rats treated orally with ^{14}C-3,3'-dimethoxybenzidine excreted ~35% of the dose of radioactivity in the urine and ~50% in the feces in 3-8 days (Bowman et al., 1982; Rodgers et al., 1983). Experiments in bile-exteriorized rats treated intravenously with ^{14}C-3,3'-dimethoxybenzidine indicated that

biliary excretion of radioactivity was extensive and accounted for most of the fecal excretion. Of the biliary radioactivity excreted ~33% was reabsorbed. Therefore, absorption of 3,3'-dimethoxybenzidine by the gastrointestinal tract was extensive. Rats treated dermally with ^{14}C-3,3'-dimethoxybenzidine absorbed ~28% of the applied dose of radioactivity at a half-life of 52.9 hours (Shah and Guthrie, 1983). The appearance of 3,3'-dimethoxybenzidine in the urine of workers occupationally exposed indicates that dermal absorption also occurs in humans (Meigs et al., 1954).

Following absorption, 3,3'-dimethoxybenzidine and its metabolites are widely distributed to tissues and organs, with the highest concentrations beubg in the liver and gastrointestinal tract; this was probably due to biliary excretion and enterohepatic recirculation (Bowman et al., 1982; Rodgers et al., 1983; Shah and Guthrie, 1983). 3,3'-Dimethoxybenzidine was metabolized by N-acetylation, hydroxylation, O-demethylation and glucuronidation in rats (Rodgers et al., 1983). Unchanged parent compound was excreted in the urine and metabolites were excreted in the urine, feces and bile.

5. EFFECTS

5.1. CARCINOGENICITY

5.1.1. Animal Studies. Hadidian et al. (1968) gave groups of six (three male and three female) Fischer rats doses of 0.1, 0.3, 1.0, 3.0 and 30 mg/rat/day of pure 3,3'-dimethoxybenzidine and one group of 29 (14 male and 15 female) Fischer rats 10 mg/rat/day of the compound in steroid-suspending vehicle (sodium chloride, sodium carboxymethylcellulose, polysorbate 80, benzyl alchohol and water) by gavage 5 times/week for 52 weeks followed by a 6-month observation period. A group of 480 vehicle control rats received steroid-suspending vehicle only by gavage 5 days/week, and a group of 180 rats was maintained as untreated controls. Limited histopathological examinations were performed. Grossly abnormal tissues were examined and a random sample of 25% of grossly normal tissues were examined microscopically. No tumors were reported in female rats given 0.1 and 0.3 mg of 3,3'-dimethoxybenzidine. A total of eight tumors were found in the rats given 1 and 3 mg, and 19 tumors were observed in the 29 rats given 10 mg doses. At 30 mg, six rats had five tumors. The tumors occurred at various sites and included bladder (two papillomas), intestine (three carcinomas), skin (five carcinomas) and Zymbal gland (three carcinomas) tumors. None of these tumor types were found in vehicle (480) and untreated (180) controls; however, since the control data were not adequately reported, the significance of the results in the treated rats cannot be readily assessed. IARC (1974) concluded that this study indicated that 3,3'-dimethoxybenzidine had a carcinogenic effect in animals. Preliminary results of this study were reported by Weisburger et al. (1967).

Saffioti et al. (1967) fed groups of 30 male and 30 female 9-week-old Syrian golden hamsters a 0.1% concentration of 3,3'-dimethoxybenzidine for their lifetimes. Controls were given feed without 3,3'-dimethoxybenzidine.

Based upon an observed weekly food intake of 60 g/animal the authors estimated an ingestion of ~60 mg/hamster/week. The average weight of the hamsters was not reported. All tumors and suspicious gross lesions were examined histologically, as well as the bladders, most of the livers, kidneys, adrenals and some additional organs. One male hamster that died at week 144 had a small transitional cell carcinoma of the bladder (Table 5-1). The authors considered the bladder tumor to be treatment-related because it was a rare tumor type that had never appeared in "several hundred" historical control hamsters from the same laboratory. Sellakumar et al. (1969) reported a similar study using hamsters that were given a concentration of 1.0% 3,3'-dimethoxybenzidine in the diet. The incidence of forestomach papillomas increased to 37% in treated hamsters compared with 2% in the controls. [OSHA/NIOSH (1980) reported that a personal communication from the authors corrected the incidence to 57%.] Few details were provided in this brief abstract. Group size was not specifically reported but was stated to be similar to that in the Saffioti et al. (1967) study. The data are summarized in Table 5-2.

Pliss (1963, 1965) gave unspecified numbers of rats of both sexes (weighing 100-130 g) 30 mg of 3,3'-dimethoxybenzidine (purity unspecified) in sunflower seed oil by stomach tube 3 times/week for 13 months (Table 5-3). A group of 50 rats were maintained as controls; half were untreated and half were injected subcutaneously with sunflower seed oil. Histopathological examinations were performed, but organs and tissues sampled were not specified. Four of 18 surviving rats had tumors; two had Zymbal gland tumors, one had an ovarian tumor and another had a fibroadenoma of the mammary gland. None of these tumor types were found in controls. IARC

TABLE 5-1

Incidence of Bladder Carcinoma in a Group of 30 Nine-Week-Old Male Syrian Golden Hamsters Given 3,3'-Dimethoxybenzidine of Unspecified Purity in the Diet for Their Lifespan*

Concentration	Tumor Incidence
0	0/30
0.1%	1/30

QUALITY OF EVIDENCE

Strengths of Study:	Both sexes of one species were exposed to 3,3'-dimethoxybenzidine for their lifetime by a relevant route of administration.
Weaknesses of Study:	Only one dose level was tested. Whether or not the MTD was estimated by adequate pretesting was not reported. Only limited histopathological examination of experimental animals was performed.
Overall Adequacy:	Limited.
Comment:	Bladder tumors were not seen in "several hundred" historical control hamsters from this laboratory

*Source: Saffioti et al., 1967

TABLE 5-2

Incidence of Papillomas of the Forestomach in Male and Female (Combined) Syrian Golden Hamsters Given 3,3'-Dimethoxybenzidine in the Diet for Their Lifespan*

Concentration	Tumor Incidence
0	1/60 (2%)
1.0%	34/60 (57%)

QUALITY OF EVIDENCE

Strengths of Study: Both sexes were treated by a relevant route for their lifespan at the MTD.

Weakness of Study: Study reported in limited detail in abstract form so that extent of histological examination was not stated. Incidence were reported for males and females combined.

*Source: Sellakumar et al., 1969; OSHA/NIOSH, 1980

TABLE 5-3

Tumor Incidence in Rats of Both Sexes Given
3,3'-Dimethoxybenzidine of Unspecified Purity in Sunflower Seed
Oil by Stomach Tube, 3 Times/Week for 13 Months*

Sex	Dose (mg)	Target Organ	Tumor Type	Tumor Incidence
NR	30	Zymbal gland	NR	2/18
NR	0	Zymbal gland	NR	0/50
NR	30	mammary gland	fibroadenoma	1/18
NR	0	mammary gland	fibroadenoma	0/50
F	30	ovary	NR	1/18
F	0	ovary	NR	0/50

QUALITY OF EVIDENCE

Strengths of Study: Both sexes of one species were tested by a relevant route of administrations.

Weakness of Study: The duration of the study was not comparable to the lifespan of the animal. Only one dose was tested and whether or not this dose was at or near the MTD was not reported. The data were not statistically analyzed.

*Source: Pliss, 1963, 1965; IARC, 1974

NR = Not reported

(1982) concluded that these data provided sufficient evidence that 3,3'-dimethoxybenzidine is carcinogenic to animals. 3,3'-Dimethoxybenzidine is currently being studied for carcinogenicity in a chronic drinking water study in rats (NTP, 1986).

5.1.2. Human Epidemiological Studies. Kuratsune and Takemura (1969) reported 23 cases of urinary system cancer in Japanese workers exposed to benzidine, 3,3'-dimethoxybenzidine and 3,3-dimethylbenzidine. Benzidine is a known human carcinogen and the data in this study are insufficeint to assess the carcinogenicity of the disubstituted benzidines. The only other detail provided was that 14 of the workers had been exposed for 6 years. Reinhardt (1979) reported no evidence of carcinogenicity of 3,3'-dimethoxybenzidine in 60 humans in ~60 years of handling the chemical.

5.2. MUTAGENICITY

3,3'-Dimethoxybenzidine has produced generally positive results in mutagenicity assays. 3,3'-Dimethoxybenzidine gave positive results in the Salmonella typhimurium reverse mutation assay with Aroclor-induced rat S-9 metabolic activation in strains TA1538, TA98 and TA100 by plate incorporation (Table 5-4) (Reid et al., 1984a; Anderson and Styles, 1978; Rodgers et al., 1983). Lazear and Louie (1977) obtained negative results in a plate incorporation assay in S. typhimurium strains TA98 and TA100 using uninduced mouse S-9 activation. Reid et al. (1984b) used a modified Ames test in which 3,3'-dimethoxybenzidine was sequentially reduced and oxidized by rat cecal flora and rat S-9 with flavin mononucleotide before rat or hamster S-9. Positive results were obtained with or without use of rat cecal flora reduction system before rat S-9 activation, with or without the hamster S-9 reduction system with flavin mononucleotides present before hamster S-9 metabolic activation and with or without the presence of a rat cecal flora reduction system before hamster S-9 metabolic activation in strain TA1538.

TABLE 5-4

Mutagenicity Testing of 3,3-Dimethoxybenzidine

Assay	Indicator/ Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	S. typhimurium TA98, TA100, TA1538	98%	plate incorporation	25-100 µg/plate	Aroclor-induced rat S-9	+	Not tested in the absence of S-9	Reid et al., 1984a
Reverse mutation	S. typhimurium TA1535	98%	plate incorporation	25-100 µg/plate	Aroclor-induced rat S-9	-	Not tested in the absence of S-9	Reid et al., 1984a
Reverse mutation	S. typhimurium TA1538	>98%	plate incorporation	0-1.0 µmol/ assay[a]	Aroclor-induced rat S-9	+	Overnight reduction system with rat cecal flora[b] before exposure of bacteria	Reid et al., 1984b
Reverse mutation	S. typhimurium TA1538	>98%	plate incorporation	0-1 µmol/ assay[a]	Aroclor-induced rat S-9	+	Reduction system not used[b]	Reid et al., 1984b
Reverse mutation	S. typhimurium TA1538	>98%	plate incorporation	0-1 µmol/ assay[a]	hamster S-9	+	Hamster S-9 with flavin-mononucleotide reduction system[b]	Reid et al., 1984b
Reverse mutation	S. typhimurium TA1538	>98%	plate incorporation	0-1 µmol/ assay[a]	hamster S-9	+	Reduction system not used[b]	Reid et al., 1984b
Reverse mutation	S. typhimurium TA1538	>98%	plate incorporation	0-1 µmol/ assay[a]	hamster S-9	+	Overnight reduction system with rat cecal flora[b] before exposure of bacteria	Reid et al., 1984b
Reverse mutation	S. typhimurium TA1535,TA1538, TA98, TA100	NS	plate incorporation	4-2500 µg/plate	Aroclor-induced rat liver S-9	+	Data not reported	Anderson and Styles, 1978
Reverse mutation	S. typhimurium TA98	>98%	plate incorporation	25-100 µg/plate	±S-9	±	S-9 from Aroclor-induced rats; the metabolite acetyl-dimethoxybenzidine was + without S-9 and the mutagenicity was greatly enhanced by S-9	Rogers et al., 1983
Reverse mutation	S. typhimurium TA98, TA100	NS	plate incorporation	50-100 µg/plate	uninduced mouse S-9	-	Not tested without S-9	Lazear and Louie, 1977
SCE	Chinese hamster ovary	NS	liquid suspension	0-5000 µg/ml	Aroclor-induced +/-rat S-9	+/+	Responses replicated and confirmed in two laboratories	Galloway et al., 1985

TABLE 5-4 (cont.)

Assay	Indicator/ Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
UDS	cultured rat hepatocytes	NS	plate assay	500-1000 nmol/mL (specific dose levels not stated)	NA	+	Positive results in concentrations ranging from 500-1000 nmol/mL	Probst et al., 1981
UDS	HeLa cells	NS	liquid medium	10^{-4} to 10^{-6} M (specific dose levels not stated)	phenobarbitone induced rat liver	+	Positive at 10^{-6} M	Martin et al., 1978
Chromosome aberrations	Chinese hamster ovary	NS	liquid suspension	0-5000 µg/mL	Aroclor-induced +/-rat S-9	-	Results replicated and confirmed in two laboratories	Galloway et al., 1985
Chromosome aberrations	peripheral human lymphocyte	NS	NA	4-19 years of occupational exposure	NA	+	3.34% chromosomal aberrations for the exposed group as compared with 0.26% in the controls	Qu and Li, 1981

[a]Dose/assay = the amount of 3,3-dimethyoxybenzidine added: 1) overnight incubation mixture for the cecal bacterial reduction system (see below) and 2) to the S-9 mix for the flavin mononucleotide system (see below).

[b]The dye was sequentially reduced and oxidized by rat cecal flora or hamster S-9 supplemented with flavin mononucleotide before rat or hamster S-9 activation.

NS = Not stated; NA = not applicable

Equivocal results have been obtained in plate incorporation assays in strain TA1535 with rat S-9 metabolic activation; Reid et al. (1984a) obtained negative results, whereas Anderson and Styles (1978) obtained positive results. It is noted, however, that Reid et al. tested only up to 100 µg/plate while Anderson and Styles tested up to 2500 µg/plate. Rodgers et al. (1983) demonstrated that the metabolite acetyldimethoxybenzidine was mutagenic to TA98 in the absence of S-9, but that the mutagenicity was greatly enhanced by the presence of S-9.

Galloway et al. (1985) obtained positive results for 3,3'-dimethoxybenzidine in the SCE test but negative results for chromosome aberrations in Chinese hamster ovary cells with or without rat S-9 metabolic activation. UDS was induced in a plate assay with cultured rat hepatocytes (Probst et al., 1981) and in HeLa cells in liquid medium with metabolic activation (phenobartitone-induced rat liver) (Martin et al., 1978). Qu and Li (1981) reported an increase in chromosomal aberrations in the peripheral lymphocytes of workers occupationally exposed to 3,3'-dimethoxybenzidine, benzidine or methylnaphthylamine. Since a single figure of 3.34% was reported, it is assumed that the workers had been exposed to all three chemicals.

5.3. TERATOGENICITY

Pertinent data regarding the teratogenicity of 3,3'-dimethoxybenzidine could not be located in the available literature as cited in the Appendix.

5.4. OTHER REPRODUCTIVE EFFECTS

Pertinent data regarding the reproductive effects of 3,3'-dimethoxybenzidine could not be located in the available literature as cited in the Appendix.

5.5. CHRONIC TOXICITY

Hadidian et al. (1968) gave groups of six (three male and three female) Fischer rats doses of 0.1, 0.3, 1.0, 3.0 and 30 mg/rat/day 3,3'-dimethoxybenzidine and one group of 30 (15 male and 15 female) Fischer rats 10 mg/rat/day of the compound in a steroid suspending vehicle by gavage 5 times/week for 52 weeks followed by a 6-month observation period (see Section 5.1.). Various nonneoplastic lesions were observed at several doses. Hyperplasia of the mammary gland was observed in one of the female rats given 0.1 mg of the compound. Uterine polyps were observed in rats dosed with 0.1 mg (one rat), 1.0 mg (one rat) and 10 mg (two rats) 3,3'-dimethoxybenzidine. One liver lesion was observed in one male given 1.0 mg of 3,3'-dimethoxybenzidine, and testicular atrophy was observed in one male rat dosed with 10 mg of the compound. Inadequate reporting of control data, small sample size and lack of statistical analysis of the data precluded assessing the significance of these results.

Hadidian et al. (1968) dosed groups of three weanling male rats with 3.0, 10, 30, 100 and 300 mg/rat/day 3,3'-dimethoxybenzidine by gavage 5 days/week for 4 weeks. All three rats in the 100 and 300 mg/rat/day groups died. At 3 and 30 mg/rat/day, no rats died and at 10 mg/rat/day one rat died. Signs of toxicity included liver and kidney discoloration, sediments in the urinary bladder, gastritis, intestinal hemorrhage, no weight gain or weight loss, and general congestion of the digestive organs. No control data were reported.

5.6. OTHER RELEVANT INFORMATION

NIOSH (1986) reported an oral LD_{50} of 1920 mg/kg for rats.

5.7. SUMMARY

Rats treated by gavage with 3,3'-dimethoxybenzidine at 0.1-30 mg/rat/day, 5 days/week for 1 year and observed for an additional 6 months had a dose-related increase in total tumors (Hadidian et al., 1968). Small group sizes and incomplete reporting of control data preclude an adequate assessment of the results. Of 18 rats surviving treatment with 30 mg/rat/day, 3 days/week for 13 months, 4 developed tumors of the Zymbal gland, mammary gland or ovary (Pliss 1963, 1965). In hamsters treated at a dietary level of 0.1%, 1/30 males developed a bladder tumor, which was considered to be treatment-related because of the lack of occurrence in historic controls (Saffioti et al., 1967). Forestomach papillomas developed in 57% of hamsters treated at a dietary level of 1% compared with an incidence of 2% of controls (Sellakumar et al., 1969; OSHA/NIOSH, 1980). IARC (1982) considered these data to be sufficient evidence of carcinogenicity to animals. A chronic drinking water bioassay in rats is in progress (NTP, 1986). Epidemiology studies of 3,3'-dimethoxybenzidine alone were not available.

3'3-Dimethoxybenzidine generally has been positive in reverse mutation assays in Salmonella typhimurium with metabolic activation (Reid et al., 1984a,b; Anderson and Styles, 1978) for SCE in Chinese hamster ovary cells with and without metabolic activation (Galloway et al., 1985) for UDS in cultured rat hepatocytes (Probst et al., 1981) and HeLa cells (Martin et al., 1978).

Pertinent data regarding the teratogenicity and reproductive effects of 3,3'-dimethoxybenzidine could not be located in the available literature as cited in the Appendix.

The toxicity of 3,3'-dimethoxybenzidine has not been adequately studied. Nonneoplastic lesions in the mammary gland, uterus, liver and testes were observed in rats treated by gavage with 0.1-30 mg/rat/day, 5 days/week for 52 weeks and observed for 6 months (Hadidian et al., 1968). Inadequate reporting of control data, small sample size and lack of statistical analysis of the data precluded assessing the significance of these results.

Hadidian et al. (1968) dosed groups of three male rats with 3.0, 10, 30, 100 or 300 mg/rat/day of 3,3'-dimethoxybenzidine by gavage 5 days/week for 4 weeks. All rats in the 100 and 300 mg/rat/day groups died. At 3 and 30 mg/rat/day, no rats died and at 10 mg one rat died. No control data were reported.

6. AQUATIC TOXICITY

Pertinent data regarding effects of 3,3'-dimethoxybenzidine on aquatic biota could not be located in the available literature as cited in the Appendix.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

OSHA and NIOSH concluded that benzidine-based dyes are potential human carcinogens (OSHA/NIOSH, 1980). U.S. EPA (1981) decided not to require additional testing of benzidine, o-tolidine and o-dianisidine based dyes. The Consumer Product Safety Commission voted to consider the issue of banning these dyes from consumer products (U.S. EPA, 1981).

7.2. AQUATIC

Guidelines and standards for the protection of aquatic biota from the effects of 3,3'-dimethoxybenzidine could not be located in the available literature as cited in the Appendix.

8. RISK ASSESSMENT

Rats treated by gavage with 3,3'-dimethoxybenzidine at 0.1-30 mg/rat/day, 5 days/week for 1 year and observed for an additional 6 months had a dose-related increase in total tumors (Hadidian et al., 1968). Small group sizes and incomplete reporting of control data preclude an adequate assessment of the results. Of 18 rats surviving treatment with 30 mg/rat/day, 3 days/week for 13 months, 4 developed tumors of the Zymbal gland, mammary gland or ovary (Pliss 1963, 1965). In hamsters treated at a dietary level of 0.1%, 1/30 males developed a bladder tumor, which was considered to be treatment-related because of the lack of occurrence in historic control (Saffioti et al., 1967). Forestomach papillomas developed in 57% of hamsters treated at a dietary level of 1% compared with 2% of controls (Sellakumar et al., 1969; OSHA/NIOSH, 1980). IARC (1982) considered these data to be sufficient evidence of carcinogenicity to animals. A chronic drinking water bioassay in rats is in progress (NTP, 1986). Epidemiology studies of 3,3'-dimethoxybenzidine alone were not available.

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Hadidian et al. (1968) dosed groups of three male rats with 3.0, 10, 30, 100 and 300 mg/rat/day of 3,3'-dimethoxybenzidine by gavage 5 days/week for 4 weeks. All rats in the 100 and 300 mg/rat/day groups died. At 3 and 30 mg, no rats died and at 10 mg one rat died. No control data were reported.

The evidence for characterizing the carcinogenicity of 3,3'-dimethoxybenzidine from chronic animal studies is marginal and in the context of U.S. EPA's Cancer Risk Assessment Guidelines is "limited." In most cases, the number of tumors at specific sites were small and not statistically significantly different from those of controls. Some of these tumors were nevertheless considered treatment-related because of their rarity in unexposed animals. In one study, a large and apparently significant increase in the percentage of forestomach papillomas was reported. This study was, however, published only in abstract form and few details are available.

3,3'-Dimethoxybenzidine, however, is mutagenic in most test systems. Furthermore, related compounds, specifically benzidine-based compounds, have evidence for carcinogenicity with benzidine itself being a known human carcinogen. The active form of these chemicals is uncertain; therefore, it is possible that 3,3'-dimethoxybenzidine may be metabolized or otherwise be in the same active form as these carcinogens.

Although the animal studies are limited in defining carcinogenic activity because of the demonstrated mutagenicity of 3,3'-dimethoxybenzidine and because of its close structural relationship to benzidine compounds that are known carcinogens, the evidence for 3,3'-dimethoxybenzidine is upgraded to a sufficient level of animal data -- EPA Group B2. Thus, this compound is considered to be a probable human carcinogen.

OSHA/NIOSH (1980) concluded that benzidine-based dyes were potential human carcinogens. Thus, it is appropriate to derive a q_1^*; however, these studies (Hadidian et al., 1968; Saffioti et al., 1967; Sellakumar et al., 1969; Pliss, 1963, 1965) have limitations. U.S. EPA (1980) concluded that two of these studies (Hadidian et al., 1968; Pliss, 1963, 1965) "may not be of sufficient strength to support a detailed risk assessment." The Hadidian et al. (1968) study has several weaknesses: inadequate reporting of control data, small sample size, limited histopathological examination and no statistical analysis seriously impaired the ability to assess the significance of these results. The Russian studies (Pliss, 1963, 1965) also had several limitations. The rats were dosed for 10-13 months, which may not have been long enough for the appearance of late-developing tumors. In addition, only limited histopathological examinations were performed, only one dose was tested and tumor incidences were too low to be significantly increased. Although U.S. EPA (1980) did not comment on the study in hamsters by Saffioti et al. (1967), this study has weaknesses as well. Syrian golden hamsters of both sexes were given a 0.1% concentration of 3,3'-dimethoxybenzidine in the diet for their lifespan. The only tumor observed was a small transitional cell carcinoma of the bladder in an aged hamster (at least 144 weeks old). This tumor was considered to be treatment-related because this was a rare tumor type that had never occurred

in historical controls. Additional limitations of this study included the following: whether or not the MTD was estimated by adequate pre-testing was not reported; limited histopathological examination was performed; and only one concentration was tested. The 1.0% feeding study in hamsters by Sellakumar et al. (1969) was reported only in abstract form, providing few details. The incidence data were reported as 37% by Sellakumar et al. (1969), but OSHA/NIOSH (1980) reported that the authors corrected the incidence to 57% in a personal communication. The group size is assumed to be 30 males and 30 females in both the control and the treated group, as Sellakumar et al. (1969) stated that group sizes were similar to that of the Saffioti et al. (1967) study. Of the four studies, only the data of Sellakumar et al. (1969), as corrected by OSHA/NIOSH (1980), provides incidence data useful for quantitative risk assessment. The tumors were benign and incidences in males and females were combined, which ordinarily would preclude the study from consideration for q_1^* derivation. Since the chemical is considered to be carcinogenic by IARC (1974, 1982) and by OSHA/NIOSH (1980), however, it seems appropriate to derive a potency factor, and the Sellakumar et al. (1969) study provides the best estimation from the available data. Data used to derive the q_1^* are presented in Table 8-1. The transformed dose was calculated using data provided by Saffioti et al. (1967) that hamsters used in their laboratory [i.e., the laboratory of Saffioti et al. (1967) and Sellakumar et al. (1969)] consume an average of 0.06 kg of diet/hamster/week. Multiplying 0.06 kg diet by 10,000 mg chemical/kg diet (1% dietary concentration) results in a weekly consumption of test compound of 600 mg/hamster/week. Dividing by a reference weight of 0.14 kg (U.S. EPA, 1985) and 7 days, results in a dosage of 612 mg/kg/day.

TABLE 8-1

Cancer Data Sheet for Derivation of q_1^*
for 3,3'-Dimethoxybenzidine

Compound: 3,3'-Dimethoxybenzidine

Reference: Sellakumar et al., 1969; OSHA/NIOSH (1980)

Species, strain, sex: hamster, Syrian, male and female

Body weight: 0.14 kg (assumed)

Length of exposure (le) = lifespan

Length of experiment (Le) = lifespan

Tumor site and type: forestomach, papilloma

Route, vehicle: oral, diet

Experimental Doses or Exposures	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Examined
0	0	1/60
1%	612	34/60

Unadjusted q_1^* from study = 1.7798123×10^{-3} $(mg/kg/day)^{-1}$

Human q_1^* = 1.412638×10^{-2} $(mg/kg/day)^{-1}$

The unadjusted q_1^* of 1.78×10^{-3} $(mg/kg/day)^{-1}$ was calculated using the computerized multistage model developed by Howe and Crump (1982). The human q_1^* of 1.41×10^{-2} $(mg/kg/day)^{-1}$ was calculated by multiplying the unadjusted q_1^* by the cube root of the ratio of the reference human body weight (70 kg) to the reference hamster body weight (0.14 kg). The q_1^* of 1.41×10^{-2} $(mg/kg/day)^{-1}$ is recommended for estimating the increased lifetime risk of cancer associated with oral exposure to 3,3'-dimethoxybenzidine until the results of the chronic drinking water study using rats (NTP, 1986) become available. The concentrations in water associated with risk levels of 10^{-5}, 10^{-6} and 10^{-7} are 2.5×10^{-2}, 2.5×10^{-3} and 2.5×10^{-4} mg/ℓ, assuming a 70 kg man consumes 2 ℓ water/day.

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

Data are insufficient for deriving a RQ based on chronic toxicity (Table 9-1). Hadidian et al. (1968) gave groups of six (three male and three female) Fischer rats doses of 0.1, 0.3, 1.0, 3.0 and 30 mg/rat/day 3,3'-dimethoxybenzidine and one group of 30 (15 males and 15 females) Fischer rats 10 mg of the compound in steroid suspending vehicle by gavage 5 times/week for 52 weeks followed by a 6-month observation period. Nonneoplastic lesions in the mammary gland, uterus, liver and testes were observed. Inadequate reporting of control data, small sample size and lack of statistical analysis of the data precluded assessing the significance of these results. Hadidian et al. (1968) dosed groups of three male rats with 3.0, 10, 30, 100 and 300 mg/rat/day of 3,3'-dimethoxybenzidine by gavage 5 days/week for 4 weeks. All rats in the 100 and 300 mg/rat/day groups died. At 3 and 30 mg, no rats died and at the 10 mg level one rat died. No control data were reported. Since the deaths represented an acute response to a relatively high dose, it is not appropriate to calculate a CS for these data.

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

Epidemiological data regarding the carcinogenic potential of exposure to 3,3'-dimethyoxybenzidine alone were not available. According to IARC (1974, 1982) there is sufficient evidence supporting the conclusion that 3,3'-dimethoxybenzidine is an animal carcinogen by the oral route; however, the studies available have weaknesses that preclude their use for quantitative risk assessment. Hadidian et al. (1968) gave groups of six (three male and three female) Fischer rats doses of 0.1, 0.3, 1.0, 3.0 and 30 mg/rat/day of pure 3,3'-dimethoxybenzidine and one group of 29 (14 male and 15 female) Fischer rats 10 mg of the compound in steroid-suspending vehicle. No tumors

TABLE 9-1

3,3'-Dimethoxybenzidine

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:

Dose:

Effect:

Reference:

RV_d:

RV_e:

Composite Score:

RQ: Data are not sufficient for deriving an RQ

were reported in female rats given 0.1 or 1.0 mg/rat/day of 3,3'-dimethoxybenzidine. Eight tumors were found in the rats given 1 and 3 mg/rat/day, and 19 tumors were observed in the 29 rats given 10 mg doses. At 30 mg, six rats had five tumors. Tumor types observed were papillomas of the bladder and carcinoma of the intestine, skin and the Zymbal gland. This study has several weaknesses. Inadequate reporting of control data, small sample size, limited histopathological examination and no statistical analysis seriously impaired the ability to assess the significance of these results. Furthermore, although total tumor incidences were increased in a dose-related manner, the incidence of specific tumor types was low. U.S. EPA (1980) concluded that this study "may not be of sufficient strength to support a detailed risk assessment."

As reported in two Russian studies, Pliss (1963, 1965) gave rats of both sexes 30 mg/rat/day 3,3'-dimethoxybenzidine of unspecified purity by stomach tube, 3 times/week for 13 months. Four of 18 surviving animals had tumors of the Zymbal gland (2/18), mammary gland (1/18) and ovary (1/18). None of these tumors were observed in controls. This study also had several limitations. The rats were dosed for 10-13 months, which may not have been of sufficient duration to allow for late tumor development. Whether or not the MTD was adequately estimated by sufficient pretesting could not be readily discerned. Other weaknesses were that only limited histopathological examinations were performed, only one dose was tested and tumor incidences were low. U.S. EPA (1980) concluded that this study may not be of sufficient strength to support a detailed risk assessment.

U.S. EPA (1980) did not comment on the study in hamsters by Saffioti et al. (1967); however, this study has weaknesses as well. Syrian golden

hamsters of both sexes were given a 0.1% concentration of 3,3'-dimethoxybenzidine in the diet for their lifespan. The only tumor observed was a small transitional cell carcinoma of the bladder in an aged hamster (at least 144 weeks old). This tumor was considered to be treatment-related because it was a rare tumor type that had never occurred in historical controls. Additional limitations of this study were that whether or not the MTD was estimated by adequate pretesting was not reported, limited histopathological examinations were performed and only one concentration was tested.

The 1.0% feeding study in hamsters by Sellakumar et al. (1969) (see Table 5-2) was reported only in abstract form, providing few details. The incidence data were reported as 37% by Sellakumar et al. (1969), but OSHA/NIOSH (1980) reported that the authors corrected the incidence to 57% in a personal communication. The group size is assumed to be 30 males and 30 females in both the treated and the control groups, as Sellakumar et al. (1969) stated that group sizes were similar to that of the Saffioti et al. (1967) study. As discussed in Chapter 8, the data of Sellakumar et al. (1969), as corrected by OSHA/NIOSH (1980), can be used to estimate potency, despite their limitations, until the results of a chronic drinking water study using rats (NTP, 1986) become available. Data used to calculate the F factor are summarized in Table 9-2. The transformed dose was calculated as previously described in Chapter 8. The unadjusted $1/ED_{10}$ was calculated using the multistage model (Howe and Crump, 1982), and the F factor was calculated by multiplying the unadjusted $1/ED_{10}$ by the cube root of the reference human body weight to the hamster body weight. The F factor of 0.1 $(mg/kg/day)^{-1}$ is <1, placing 3,3'-dimethoxybenzidine in Potency Group 3.

TABLE 9-2

Derivation of Potency Factor (F) for 3,3'-Dimethoxybenzidine

Reference:	Sellakumar et al., 1969; OSHA/NIOSH, 1980	
Exposure route:	Oral	
Species:	Hamster	
Strain:	Syrian	
Sex:	Male and Female	
Vehicle or physical state:	Diet	
Body weight:	0.14 kg	
Duration of treatment:	Lifespan	
Duration of study:	Lifespan	
Target organ:	Forestomach	
Tumor type:	Papilloma	
Experimental doses/exposure:	0	1.0%
Transformed doses (mg/kg/day):	0	612
Tumor incidence:	1/60	34/60
Unadjusted ED_{10}:	1.2708×10^{-2} $(mg/kg/day)^{-1}$	
$1/ED_{10}$ (F factor):	0.1 $(mg/kg/day)^{-1}$	

Because human carcinogenicity data are inadequate, but the data in animals are sufficient (IARC, 1982, 1984), 3,3'-dimethoxybenzidine is an IARC Group 2B and an EPA B2 chemical, indicating that it is a probable human carcinogen. Although the animal studies are limited in defining carcinogenic activity because of the demonstrated mutagenicity of 3,3'-dimethoxybenzidine and because of its close structural relationship to benzidine compounds that are known carcinogens, the evidence for 3,3'-dimethoxybenzidine is upgraded to a sufficient level of animal data -- EPA Group B2. Thus, this compound is considered to be a probable human carcinogen. A Potency Group 3 and EPA Group B2 chemical has a LOW hazard ranking under CERCLA.

10. REFERENCES

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APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

GLOBAL
TSCATS
CASR online (U.S. EPA Chemical Activities Status Report)
CAS online STN International
TOXLINE
TOXBACK 76
TOXBACK 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register

These searches were conducted in April, 1986. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1986. Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 1985-1986. TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1985-1986. Cincinnati, OH. 114 p.

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Grayson, M. and D. Eckroth, Ed. 1978-1983. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. John Wiley and Sons, NY. 23 Volumes.

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Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY.

Windholz, M., Ed. 1983. The Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ.

Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

Battelle's Columbus Laboratories. 1971. Water Quality Criteria Data Book. Volume 3. Effects of Chemicals on Aquatic Life. Selected Data from the Literature through 1968. Prepared for the U.S. EPA under Contract No. 68-01-0007. Washington, DC.

Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. U.S. Dept. Interior, Fish and Wildlife Serv. Res. Publ. 137, Washington, DC.

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